Protection Branch Report of Test No. 19-60

Investigation of Bacterial Contamination Inside Electronic Components. Test I.

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Various electronic components, received in January 1960 from the Goddard Space Flight Laboratories, Washington, D.C., were tested for possible internal bacterial contamination.

MATERIALS AND METHODS

The testing techniques were basically the same as described in Protection Branch Report of Test No. 7-60 except for the following modifications:

Exposure to Ethylene Oxide:

The items within the test chamber were exposed to ethylene oxide for six hours instead of eight because sterilization of the external surfaces of the items and the atmosphere within the chamber can be achieved within the shorter exposure period.

Broth Blanks:

Before the broth blanks were exposed to ethylene oxide within the chamber, the rims of the metal caps of the bottles containing tryptose broth were wiped with hypochlorite solution then electricians tape was applied. The tape prevents ethylene oxide from penetrating into the broth under the cap of the bottles. The presence of ethylene oxide in the blank would inhibit the growth of the bacteria introduced. However, since the tape also prevents sterilization of the rims of the caps with ethylene oxide, decontamination of the rims before applying the tape seemed to be a

necessary precaution to prevent bacteria upon the rims from contaminating the broth when the cap is removed to place the electronic component into the broth.

Test for Sterility:

The sterility test was expanded over that which was reported earlier. In these tests the procedure was as follows: after the electronic components were exposed to ethylene oxide each was broken, ground as well as possible, and the pieces placed in a broth blank to incubate at 37 C. If a broth sample became cloudy, an aliquot was immediately streaked on tryptose agar to confirm whether or not this was due to bacterial growth. If no growth occurred the broth sample was reincubated and checked periodically until growth was evident on agar or the broth sample had incubated at least seven days. At the end of this time aliquots of these broth samples as well as aliquots of the broth samples which showed no cloudiness were streaked on agar as the final test to check bacterial growth. Each time a broth sample was checked for bacterial growth a methylene blue stain of this broth was examined microscopically for bacteria and subsequently compared with a stain of the bacteria from agar if growth occurred. If no microorganisms grew on agar or if no microorganisms were seen on methylene blue stain of the broth, the broth sample was inoculated with a dilute suspension of a 24 hour tryptose broth culture of Staphylococcus aureus (about 100 microorganisms) to assure that the broth was capable of supporting growth.

RESULTS

Whenever possible the results given in this report (Tables I-V) are according to type of component, however, different types of components were tested in the same experiment. Table I shows that six out of eight varieties of capacitors tested had internal bacterial contamination. Internal bacterial contamination was also present in one out of the five varieties of resistors tested (Table II) and in one out of the four varieties of transistors (Table III). The only component given in Table IV that had internal bacterial contamination was the output transformer. The two parts of each of the first three components given in Table V were inoculated with the 500 spores of Bacillus subtilis var niger and the reassemble to finger tightness. After they were exposed to ethylene oxide, each component was disassembled and put into a broth blank. Since all components seemed to be sterile, ethylene oxide probably can sterilize components having easily removable parts.

DISCUSSION

Up to now all testing of electronic components has been "spot testing", that is, only one or two of any one variety of component has been assayed. Even though only 30-40 individual components have been tested, it is evident that microorganisms are capable of surviving inside many types of such items. As pointed out in Protection Branch Report of Test 7-60, if one of any particular variety of component is found internally contaminated all of this variety used in payloads designated for the vicinity of Venus, Mars and the Moon will have to be either manufactured under more aseptic conditions or given a non gaseous sterilization treatment. It should be pointed out again that finding one or two of one variety of electronic component sterile should not be misconstrued as indicative that all of this variety are sterile.

Table I.

Investigation of Microbial Contamination Inside Various Capacitors

Electronic Component	Incubation of Broth (Days)	Tryptose Broth	Tryptose Agar Spread Plate	Methylene Blue Stain of Broth & Agar
1) Ceramic Capacitor, 0.1 ufD	3	Cloudy	Orange growth	Cocci in clusters
2) 4-lead disc ceramic capacitor .0022 ufD	3	Cloudy with mold on top	White growth	Large cocci in pairs and packets of 4
3) Variable capacitor, JFD VC-9G	2	Cloudy	Orange growth	Cocci in clusters
4) Midget mica capacitor, 200 uuf type 22R "Silver Mike"	1	Cloudy	White growth	Large cocci in pairs and packets of 4
5) Capacitor, 20 uuf	9*	Cloudy	No growth	No organisms
6) Capacitor, 100 uuf	1	Cloudy	White growth	Cocci in clusters
7) Tantalum capacitor, 6.8 uuf	12*	Cloudy	No growth	No organisms
8) Disc ceramic capacitor .002 ufD	3	Cloudy	Rough yellow growth	Bacilli and few spores

* Broth supported growth of the bacteria introduced after the maximum incubation period.

Table II.

Investigation of Microbial Contamination Inside Various Resistors

Electronic Component	Incubation of Broth (Days)	Tryptose Broth	Tryptose Agar Spread Plate	Methylene Blue Stain of Broth & Agar
1) Resistor, 22000 ohms, 1 watt	3	Cloudy	White growth	Cocci in clusters and bacilli
2) Resistor, 12000 ohms, 1/2 watt	7*	Cloudy	No growth	No organisms
3) Resistor, 2200 ohms, 1 watt	9*	Cloudy	No growth	No organisms
4) Variable resistor	12*	Cloudy	No growth	No organisms
5) Ceramic resistor, 30K ohms	12*	Clear	No growth	No organisms

* Broth supported growth of the bacteria introduced after the maximum incubation period.

Table III.

Investigation of Microbial Contamination Inside Various Transistors

Electronic Component	Incubation of Broth (Days)	Tryptose Broth	Tryptose Agar Spread Plate	Methylene Blue Stain of Broth & Agar
1) Transistor, 2N117, type NPN	3	Cloudy	White growth	Large cocci in pairs & packets of 4
2) Transistor, 2N383, type NPN	9*	Cloudy	No growth	No organisms
3) Transistor, 2N501, type NPN	9*	Cloudy	No growth	No organisms
4) Transistor, 2N146, type NPN	7*	Cloudy	No growth	No organisms

* Broth supported growth of the bacteria introduced after the maximum incubation period.

Table IV.

Investigation of Microbial Contamination Inside Various Electronic Components

Electronic Component	Incubation of Broth (Days)	Tryptose Broth	Tryptose Agar Spread Plate	Methylene Blue Stain of Broth & Agar
1) Radio Frequency choke (coil) Brown	9*	Cloudy	No growth	No organisms
2) Radio Frequency choke (coil) Orange	12*	Cloudy	No growth	No organisms
3) Diode-Yellow	13*	Cloudy	No growth	No organisms
4) Diode-Blue	9*	Clear	No growth	No organisms
5) Quartz crystal M-20	9*	Cloudy	No growth	No organisms
6) Output transformer, 600 ohms 3MA, 3.2 ohms	3	Cloudy	White growth	Bacilli and spores
7) Thermistor	9*	Slightly cloudy	No growth	No organisms

* Broth supported growth of the bacteria introduced after the maximum incubation period.

Table V.

Sterilization of Various Electronic Components Having Parts Contaminated with Bacterial Spores

Electronic Component	Incubation of Broth (Days)	Tryptose Broth	Tryptose Agar Spread Plate	Methylene Blue Stain of Broth & Agar
1) Microdot Connector	11*	Clear	No growth	No organisms
2) Heat sink	9*	Slightly cloudy	No growth	No organisms
3) Amphenol, Blue Ribbon Connector	9**	Clear	No growth	No organisms
4) Mylar Magnetic Tape	13*	Clear	No growth	No organisms

* Broth supported growth of the bacteria introduced after the maximum period.

** Broth did not support growth of the bacteria introduced after the maximum incubation period.